

Occurrence of *Xylella fastidiosa* in Texas Sharpshooter Populations

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Pierce's Disease (PD) of grapes

- Identified in California in 1876 (Pierce)
- The Cost:
 - 1.1 million acres of grapes possibly at risk
 - California grape industry, \$3.3 Billion



Chlorosis, Necrosis



Vine death within 3-4 years

Grapevine



Marked for Removal

- Reduced production from infected vines
- 5-7 years before replacement vines are fully productive
- Up to 10 years of lost yield due to infection

The Texas Wine Grape Industry

The Numbers

- 210 commercial vineyards
- approximately 3,200 acres
- \$38.4 million

Growth of the Industry

- 14,000 gallons in 1979
- 1.6 million gallons in 1997



<http://www.agr.state.tx.us/wine/docs/introduction.htm>

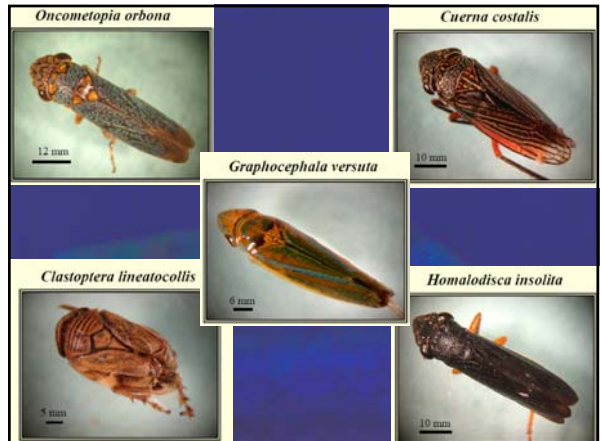
Adult



- The Glassy Winged Sharpshooter (GWSS)
- *Homalodisca coagulata* Say
- Hemiptera: Cicadellidae

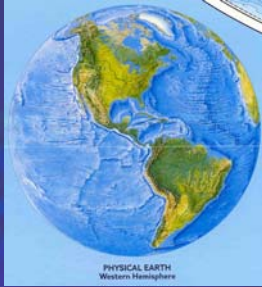


Eggs



Strain Diversity

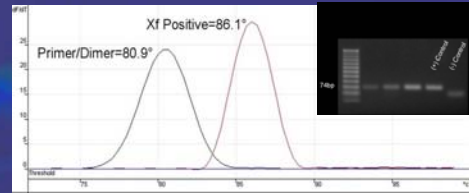
1. **Pierce's disease** (subsp. *piercei*)
2. **Almond leaf scorch** (Dixon-like ALS)
3. **East Coast™** (subsp. *multiplex*)
4. **Oleander leaf scorch (OLS)**
5. **"Citrus Variegated Chlorosis"** (subsp. *pauca*)



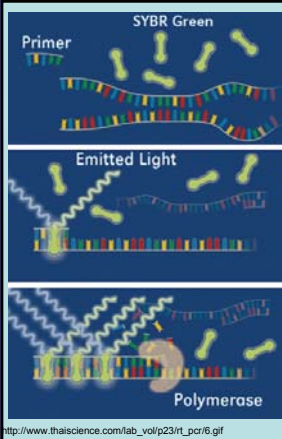
*subsp. from Schaad et al. 2004

Optimizing Real-time PCR Diagnostics:

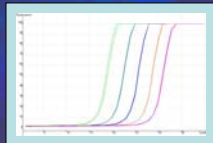
SYBR® Green-based Strain Diversity



How SYBR Green® Works



<http://www.deborahataylor.net/images/eybr.jpg>



Strain Diversity based on amplicon Tm

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PD (Tamecula) 1  aggttagaggtgcagopaaacgggtactcaatttcggttttaaacocypcaaaagagatcttcagtgat 70
ALS (Dixon) 1  agtttagaggtgcagopaaacgggtactcaatttcggttttaaacocypcaaaagagatcttcagtgat 70
OLS (ANN) 1  agtttagaggtgcagopaaacgggtactcaatttcggttttaaacocypcaaaagagatcttcagtgat 70

PD (Tamecula) 71  gggagttcattacgaaactctgaaagocgctocgtaggtatctctctcaattccggcctaaagt 140
ALS (Dixon) 71  gggagttcattacgaaactctgaaagocgctocgtaggtatctctctcaattccggcctaaagt 140
OLS (ANN) 71  gggagttcattacgaaactctgaaagocgctocgtaggtatctctctcaattccggcctaaagt 140

PD (Tamecula) 141  agtttgatgagaaocggtggaggtgggggagcagatcttcaactgaaagtgatcttgatgcttt 210
ALS (Dixon) 141  agtttgatgagaaocggtggaggtgggggagcagatcttcaactgaaagtgatcttgatgcttt 210
OLS (ANN) 141  agtttgatgagaaocggtggaggtgggggagcagatcttcaactgaaagtgatcttgatgcttt 210

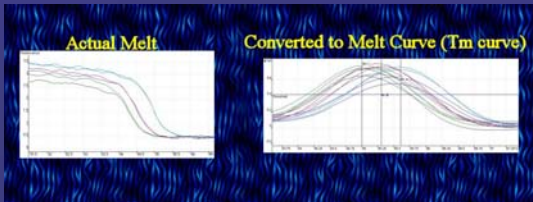
PD (Tamecula) 211  gtagacattttgctcaattgaagaccctctgcatctgataattcttggctacaggtgagcaacg 280
ALS (Dixon) 211  gtagacattttgctcaattgaagaccctctgcatctgataattcttggctacaggtgagcaacg 280
OLS (ANN) 211  gtagacattttgctcaattgaagaccctctgcatctgataattcttggctacaggtgagcaacg 280

PD (Tamecula) 281  gcaattgttgagatg-cgcttaccagtcgctgctcaccagaacaaatgatctgtttaccacata 350
ALS (Dixon) 281  gcaattgttgagatg-cgcttaccagtcgctgctcaccagaacaaatgatctgtttaccacata 350
OLS (ANN) 281  gcaattgttgagatg-cgcttaccagtcgctgctcaccagaacaaatgatctgtttaccacata 350

PD (Tamecula) 351  ttctccacaaaagatg-ggtaccacactctgctgctctcctgctgatactgactatg-ctggtaat 420
ALS (Dixon) 351  ttctccacaaaagatg-ggtaccacactctgctgctctcctgctgatactgactatg-ctggtaat 420
OLS (ANN) 351  ttctccacaaaagatg-ggtaccacactctgctgctctcctgctgatactgactatg-ctggtaat 420

PD (Tamecula) 421  tcaatggagcaaatggg-ttgctaggaagagagatcactctttctggatgagatgctgtagg 488
ALS (Dixon) 421  tcaatggagcaaatggg-ttgctaggaagagagatcactctttctggatgagatgctgtagg 488
OLS (ANN) 421  tcaatggagcaaatggg-ttgctaggaagagagatcactctttctggatgagatgctgtagg 488
    
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Strain Diversity based on amplicon Tm



Tm curve is based on the point of greatest complimentary strand separation
Greatest influencing factors: CG content and Amplicon length

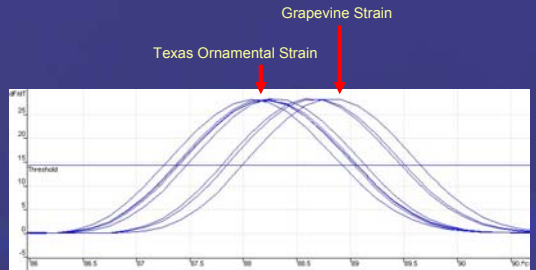
Success!
Correct ID
of
California
Strains

Strain	Collection Host	Set 6 Tm Placement	Phylogenetic Placement
103	Grapevine	PD	PD
237	Almond	PD	Not Placed
PD-Conn Creek	Grapevine	PD	PD
PD-STL	Grapevine	PD	PD
PD-Traver	Grapevine	PD	PD
ALS-Tulare ¹	Almond	PD	PD
ALS-#11	Almond	PD	PD
ALS-Fresno ²	Almond	PD	PD
ALS-#3	Almond	ALS	ALS
ALS-#6	Almond	ALS	ALS
ALS-#9	Almond	ALS	ALS
ALS-Dixon	Almond	ALS	ALS
187	Almond	ALS	Not Placed
276	Almond	ALS	ALS
OLS-TSS	Oleander	OLS	Not Placed
OLS-Texas	Oleander	OLS	Not Placed
OLS-Cathedral City	Oleander	OLS	OLS
OLS-Riverside	Oleander	OLS	Not Placed
OLS-TR2	Oleander	OLS	Not Placed
OLS-ANN-1	Oleander	OLS	OLS

The Texas Strains

- We find two main strains occurring in Texas.
 - A PD-Strain with 100% Identity to the Temecula type strain.
 - An ornamental strain that groups with the “east coast” type strains.

Melting Curve Analysis for Xf Strain Identification



We are working on getting Strain ID on these samples, but are not ready to present the data yet.

Texas Samples

- *Clastoptera sp.* 33%
- *Cuerna costalis* 0%
- *Graphocephala versuta* 45%
- *Homalodisca coagulata* 37%

- Early Season GWSS 0%
- Late Season GWSS 80%

Conclusions

- More Xf-positive insects late in the season.
- Melting curve analysis data is consistent with:
 - *H. coagulata* carrying the PD-strain
 - *G. versuta* carrying the non-PD strain.



Acknowledgements

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- Lisa Morano (U of Houston)
- Forrest Mitchell Lab Group (TAMU-Stephenville)